Abstracts

Oral Session I - Retrovirus Infections I

1

Combinatorial Libraries as Tools in Drug-Discovery: Identification of a Tat/TAR Inhibitor that Suppresses HIV-1 Replication

Thomas Klimkait, Gerd Heizmann, Eduard Felder, Gabriele Varani*, Jonathan Karn*, Janis Lazdins, François Hamy
Pharmaceutical Research, Ciba Geigy, Ltd., Basle, Switzerland;
**Laboratory of Molecular Biology, Medical Research Council, Cambridge, UK*

Chemical, amino acid related building blocks were combined in a semi-rational effort to form a large peptide-related library of synthetic entities. The goal was to target the unique, molecular interaction of HIV-1 Tat with its essential, cognate substrate RNA (TAR) for the identification and characterization of selective inhibitors. *In vitro* interaction of protein/RNA was evaluated by electromobility shift assays; NMR was used to specify the exact location of the compound interaction with the target RNA; a novel cellular reporter assay addressed cellular activity and specificity of compounds. Infectivity assays were performed in primary human PBL. Out of the original biased library containing $3.2x10^6$ compounds, a potent and selective compound could be identified *in vitro* by sequential deconvolution using gel shift assays. The resulting products, N-substituted Glycins with a M_r of <2000, possess a high proteolytic stability. Competition experiments suggest that the most potent compound binds TAR with equal affinity as the authentic Tat protein. NMR analysis demonstrates that the binding to RNA is similar to that of the native protein. A stable, HIV LTR-based cellular reporter system confirms a specific and potent inhibition of Tat/TAR-dependent gene expression in the absence of cytotoxicity. No effect on cell fusion was noted. Furthermore, at non toxic concentration full suppression of HIV-1 infection in human leukocytes is achieved by simple addition of the compound to the cell culture medium.

Our findings strongly suggest that peptidic inhibitors can be modified and optimized to serve as proteolytically stable, potent inhibitors of HIV. The demonstration of specific antiviral activity based on HIV-1 Tat inhibition encourages the use of regulatory HIV genes as novel targets for chemotherapy and antiviral intervention in AIDS.

2

Anti-HIV Agent 1592U89 Succinate - Progress from Synthesis through Clinical Development. S. M. Daluge, S. W. LaFon, S. S. Good, M. H. St. Clair., D. A. Livingston, M. T. Martin. Glaxo Wellcome, Research Triangle Park, NC, 27709, USA.

(1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol succinate (1592U89) is a carbocyclic nucleoside HIV-RT inhibitor with *in vitro* potency against clinical isolates of HIV-1 (average IC_{50} value 0.26 μM in PBLs) equivalent to that of Zidovudine (ZDV). A Phase II study in nineteen HIV-infected subjects (16 male, 3 female) with initial CD4 cell counts between 200-500/mm^3 and <12 weeks prior ZDV therapy is ongoing. Preliminary data on viral load and CD4 cell count changes (M. Saag, el al., 3rd Conference on Retroviruses and Opportunistic Infections, January 1996, Washington, D. C.) have demonstrated an antiviral effect which is better than that anticipated from *in vitro* potency comparisons to other anti-HIV agents. A practical, scalable synthesis of drug is required to support continued clinical development. Efficient, convergent syntheses have been developed utilizing novel intermediates. (1R,4S)-2-Azabicyclo[2.2.1]hept-5-en-3-one or its racemate was converted in high yield to key intermediate (1S,4R)-4-amino-2-cyclopentene-1-methanol. This aminoalcohol was condensed directly with novel N-(2-amino-4,6-dichloro-5-pyrimidinyl)formamide, bypassing problematic steps in other routes to carbocyclic purine nucleosides. The resulting 5-formamidopyrimidine adduct was cyclized by treatment with base (e.g. cyclopropylamine) or by acid-catalyzed dehydration followed by amination, providing 1592U89 in excellent overall yield and purity. N-(2-amino-4,6-dichloro-5-pyrimidinyl)formamide was synthesized in high yield from readily available starting materials and is also proving useful in the preparation of a variety of other 2-aminopurines, including metabolites and analogs of 1592U89.